THIRD QUARTERLY SUMMARY REPORT OF PROGRESS

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REDUCTION OF BACTERIAL DISSEMINATION

GERMICIDAL ACTIVITY OF ETHYLENE OXIDE

REDUCTION OF BACTERIAL CONTAMINATION ON SURFACES

by the

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Reduction of Bacterial Dissemination -- At the present time, a study is being conducted comparing the effects of showering with two different types of cleaning agents. The purpose of this series of experiments is to determine characteristics of shedding of bacterial populations from the skin of adult males who, in the showering process, have used a conventional bath soap (Ivory[1]) without a specific antimicrobial agent and a germicidal detergent (Phisohex[1]) containing hexachlorophene for reduction of skin flora.

The subjects shower each evening using one or the other of the cleaning agents. After showering, no deodorant is used. Clean street clothes are donned and normal activities are carried out until the individual is subjected to the test procedure. Approximately 12 hours after showering, the subject is dressed in a sterile surgical scrub suit, cap, and socks, and then confined in the microbiotank for 30 minutes. Following the 30-minute period, the subject emerges from the tank, and the organisms seeded in the tank are recovered in the usual manner.

The bacterial shedding from each subject is studied for five consecutive days. For the subsequent 9 days, the individual continues to shower with the same type of cleaning agent used during the initial 5 days; however, he is not placed in the microbiotank for determination of shedding

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[1] Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U. S. Department of Health, Education, and Welfare.

during this period. Then, during the following 5 days, recovery procedures for assaying the shedding rate are carried out with the individual again confined in the microbiotank for 30-minute intervals.

On the initial and final days of the 3-week experimental period, qualitative as well as quantitative characteristics of the bacterial population shed are determined. The qualitative determinations include identification of coagulase-positive staphylococci and coliforms.

These studies are presently in progress, and preliminary results indicate little reduction of the bacterial populations shed by individuals using the conventional soap without hexachlorophene.

The results with one subject are shown in table 1. The counts of

Table 1

Number of bacteria shed per minute by an individual during the period when a conventional bath soap was used during showering and during the period when a germicidal detergent was used.

Time of Sampling	Counts for Conventional Soap (Bacterial Particles per Minute)	Counts for Germicidal Detergent (Bacterial Particles per Minute)
Initial 5-day period	5,070 3,330 6,100 ---	83 203 800 160
Final 5-day period	29,300 2,200 2,830 1,670	66 220 -- --

bacteria obtained during the period when showering was carried out with a conventional bath soap ranged from 1,670 to 29,300 particles shed per minute. With the exception of one count of 29,300, the organism shed during the final 5-day period that the conventional soap was used appear to be slightly lower than those obtained during the initial period. Due to the small number of satisfactory trials, it is not possible to consider this an actual difference.

The counts obtained from the same subject during the period when a germicidal detergent was used for showering are markedly lower than those obtained when conventional soap was used. The recoveries ranged from 66 to 800 bacterial particles shed per minute, indicating that this type of cleaning agent apparently was effective in reducing the number of organisms shed by the individual. Sufficient data are not available to demonstrate whether the counts during the final 5-day period differed from those during the initial 5 days.

Germicidal Activity of Ethylene Oxide -- The study concerned with action of ethylene oxide gas against dust particulates laden with spores of B. globigii was continued utilizing a static system for exposing contaminated glass surfaces. Approximately 200 mg. of spore-laden institutional dust was nebulized into a settling chamber maintained at average intramural conditions. Glass plates, 1"x3" in size, were exposed to the settling aerosol for a period of 30 minutes after nebulization. With this exposure interval, the individual slides were loaded with an average

concentration of 2.3×10^3 spore-bearing particulates per individual plate. This quantity of contamination, 1.1×10^5 per square foot of surface, is somewhat less than the 1×10^6 bacterial-spore population reported in other studies as an estimate of various types of spores per square foot of surface under natural conditions.

Immediately following deposition of contamination on the glass surfaces, the plates were placed into a chamber and the dust preconditioned for a period of 1 hour at a vacuum of approximately 50 mm Hg absolute and relative humidity ranging from 50 to 83 percent. The contamination was then immediately exposed to a gaseous ethylene oxide-freon 12 mixture containing 12% ethylene oxide by weight. Survival of spores of B. globigii in the dust was determined by culturing the plates either in tryptose broth or tryptose agar at 37° C for periods ranging from 7 to 31 days.

A preliminary series of experiments was carried out utilizing 540 to 610 mg/liter ethylene oxide concentrations. The glass plates were exposed at atmospheric pressure for a period of 1 hour at temperatures ranging from 25 to 37° C and relative humidities between 50 and 83%. This set of conditions failed to yield a satisfactory reduction of the spore population. With a total of 44 contaminated plates exposed to the gas, viable spores of B. globigii were recovered from 41 of the plates.

In view of these results, the duration of the exposure period was increased. The contaminated glass surfaces were exposed to ethylene

oxide gas for 3 hours at atmospheric pressure immediately following a 1-hour preconditioning period under an initial vacuum of 50 mm Hg absolute. The temperatures, relative humidities, concentrations of ethylene oxide and the results of culturing exposed surfaces are shown in table 2.

Concentration of ethylene oxide gas, as measured indirectly by the weight of the gas, varied from 480 to 640 mg/liter for the various trials. Temperatures for the preconditioning period and the sterilization period ranged from 29 to 41° C and 32 to 42° C, respectively. Relative humidities for rehydration of the spores during the preconditioning period ranged from 52 percent to 83 percent. During the subsequent sterilization cycles, relative humidities varied from 56 to 92 percent.

The results show that a high level of kill was achieved under these conditions; however, a small percent of the slides yielded growth when incubated in nutrient medium. As shown in table 2, growth of B. globigii occurred from a single glass plate in Trial D and in Trial H. Viable contamination was found on two plates in Trial E. All of the trials were conducted with aliquots of contaminated dust from the same batch, and it is unlikely that the failure to sterilize was due to a marked difference of the organisms exposed. Also, each trial was performed on a different day, so it is unlikely that the results may have been due to an abnormal condition occurring during a particular day's activities. There is also no reason to consider that the supply

Table 2

Number of glass plates yielding growth of B. globigii after an exposure of 3 hours to gaseous ethylene oxide. Individual plates were inoculated with an average of 2.3×10^3 spore-laden dust particulates conditioned for 1 hour at an initial vacuum of 50 mm Hg absolute pressure prior to exposure period.

Trial	Concentration of Ethylene oxide (Mg/Liter)	Temperature		Relative Humidity		Total Number of Plates Exposed	Total Number of Plates Yielding Growth
		Conditioning Period (°C)	Sterilization Period (°C)	Conditioning Period (Percent)	Sterilization Period (Percent)		
A	480	34	36	78	86	12	0
B	530	38	38	52	72	12	0
C	530	32	36	71	86	12	0
D	530	36	43	63	62	12	1
E	560	31	32	66	84	12	2*
F	560	33	36	83	87	12	0
G	560	41	42	57	56	12	0
H	590	29	32	80	86	12	1
I	610	33	35	68	70	6	0
J	610	31	32	68	90	6	0
K	640	34	36	80	92	12	0

*Viable contamination.

of gas to the chamber may have varied between trials; as stated previously, the gas concentration was determined indirectly from the weight of gas introduced into the chamber. Consideration of the various factors in the procedure does not reveal any reason for the failure of the ethylene oxide to affect all the surfaces exposed to the gas.

The variations in temperature and humidity between trials were due to difficulties in maintaining adequate control of the conditions in the chamber. Although the equipment appeared adequate prior to use, various problems were encountered, primarily as result of malfunctioning of controls and sensing and measuring units. The limited results obtained in these studies indicate that complete control and monitoring of the conditions of temperature, humidity, and gas concentration are necessary to ensure that adequate and dependable cycles of sterilization are achieved.

Although sterilization studies will be continued with this system, interpretation of results showing growth of organisms after ethylene oxide exposure will require more refined equipment to determine whether the survival is due to mechanical or biological factors. A system providing more precise control of sterilization cycles is being assembled to determine more precisely the parameters and conditions involved in the process.

Reduction of Bacterial Contamination on Surfaces -- Surfaces of materials of different composition were contaminated by impression of small squares (about 1.5 cm^2) onto an agar surface previously seeded with a trypticase soy broth suspension of the organism. The contaminated squares were placed directly in the test atmosphere, and five squares removed at various time intervals for assay of surviving cells. Squares were mechanically shaken for 15 minutes in trypticase soy broth and plated in trypticase soy agar. All cultures were incubated at 37° C.

The suspensions of bacteria (Salmonella derby and Escherichia coli) for contaminating the agar surface were prepared from an 18-hour culture in trypticase soy broth (37° C), centrifuged, supernatant decanted, and cells resuspended in sterile trypticase soy broth. Cells were then mechanically dispersed by shaking the tube 15 minutes on a mechanical shaker. Initial counts of viable cells were made before seeding an agar surface (agar, 1.5 percent; pH 7.0, phosphate buffer) and distributing the cells evenly over the surface with a bend glass rod. Seeded plates were dried in a 37° C incubator for 20 minutes. The agar was contained in 150-mm diameter Petri dishes with a design of scored squares (about 4 cm^2) on the bottom of the dish. Each scored area was assigned a random number designating a site of impression. Sterile test material squares were placed on random sites, pressed evenly, and removed with forceps to a Petri dish. Agar squares (1 cm^2) were removed for assay of original levels of contamination.

Five agar squares and five test-material squares were used for each determination.

Contamination of surfaces of agar and of test materials with spores of Bacillus globigii was done in the same manner. A stock suspension of frozen, heat-shocked spores was thawed under running tap-water. The undiluted, thawed suspension of spores was used to inoculate the agar surface. Trypticase soy broth at 37° C was used as the culture medium to demonstrate viable cells.

A test atmosphere of 50° C and 40 percent relative humidity was maintained in a dynamic airflow chamber. A static system was used for two other sets of conditions, 50° C and 48 percent relative humidity using a saturated solution of ammonium nitrate, and 30° C and 11 to 13 percent relative humidity with a saturated solution of lithium chloride. Determinations of temperature and relative humidity were made with Hygrodynamics high-sensitivity, narrow-range sensing elements, Type TH[1].

Results of exposure of Bacillus globigii are shown in table 3. After an initial reduction during the first 4-hour period of exposure, there was a very low rate of loss of viability during the remaining 164 hours of exposure. Comparative results of loss in spore viability as a function of the material of the contaminated surface does not indicate a difference between vinyl, glass, metal (stainless steel), or ceramic tile.

Table 3

Survival of *Bacillus globigii* on Various Surfaces Exposed to 50° C and 40% R.H.

Exposure Time (Hours)	Viable Cells per Square Centimeter			
	Vinyl	Glass	Metal*	Ceramic
0	35.5×10^5	15.4×10^5	18.5×10^5	9.9×10^5
4	71.8×10^3	188×10^3	61.4×10^3	124×10^3
24	56.1×10^3	47.2×10^3	102×10^3	92.8×10^3
48	22.8×10^3	--	27.5×10^3	32.8×10^3
72	24.3×10^3	20.4×10^3	41.3×10^3	63.2×10^3
96	21.6×10^3	102×10^3	56.5×10^3	66.0×10^3
168	14.5×10^3	15.9×10^3	19.5×10^3	17.8×10^3

*Stainless Steel

In table 4, a comparison is made between the numerical density of spores per cm^2 on the agar surface and the numbers of spores adhering to the test-material surfaces following impression of the squares onto the agar surface. The different test materials were considered to have had equivalent levels of contamination at the beginning of the test (0 hours).

Table 4

Comparison of Numbers of Spores Adhering to Test-Material Surfaces Impressed onto Contaminated Agar Surface.

Viable Cells x 10^{-5} per Square Centimeter							
Agar:	60.5	Agar:	67.0	Agar:	64.5	Agar:	51.0
Vinyl:	35.5	Glass:	15.4	Metal*:	18.5	Ceramic:	9.9
Percent Adhering:	58.7		23.0		28.7		19.4

*Stainless Steel

Analysis of loss of viability of cells of S. derby and E. coli was done by plotting the surviving cell numbers on a semi-logarithmic scale and determining visually the slope, k(death rate), of the tangent to the line of best fit at different times of exposure up to 4 hours. Results reported in table 5 show a high rate of loss of viability

Table 5

Death Rates (k) of S. derby and E. coli on Different Surfaces at 50° C and 40% Relative Humidity

Organism	Surface	k Values					Viable Cells per Square Centimeter	
		0.5 Hours	1.0 Hours	2.0 Hours	3.0 Hours	4.0 Hours	0 Hours	24 Hours
E. coli	SS(*)	5.38	0.83	0.42	0.30	0.25	152×10^5	28
		5.14	0.77	0.26	0.13	0.06	145×10^5	ND(**)
S. derby	SS	5.14	0.77	0.31	0.21	0.10	472×10^5	ND(**)
		4.79	0.40	0.12	0.06	0.05	50×10^5	154
S. derby	Vinyl	2.61	0.79	0.29	0.11	0.07	213×10^3	380
		6.00	0.96	0.42	0.30	0.22	118×10^6	160
		$k_{(\bar{X})}$ 4.84	0.75	0.30	0.19	0.13		

(*) Stainless Steel
(**) Not determined

during the first 0.5 hour of exposure, followed by a progressively decreased death rate during the 0.5- to 4-hour exposure period. Viable cells of S. derby and E. coli were recovered from stainless-steel and vinyl surfaces at the end of 24 hours at 50° C and 40% relative humidity.

Experiments are in progress to evaluate the variation in recovery of cells of S. derby and E. coli from surfaces of different materials as a function of time of storage. The number of squares of test material was increased to 13 per sample set for variance analysis. An exposure atmosphere of 30° C and 11 to 13 percent relative humidity was selected as less injurious than the condition of 50° C and 40 percent relative humidity. Preliminary results indicate increased intra-sample variation at 1 hour of exposure to the test atmosphere over the variation found at $\frac{1}{2}$ hour of exposure. Earlier experiments for determining survival of S. derby and E. coli at 50° C and 40 percent relative humidity produced results of unpredictable recovery after exposure in repeated experiments, as shown in the comparison of k values in table 5. Reproducibility of results in repeated experiments is essential in establishing accurate die-away rates of organisms under storage conditions. Whether this variation is a function of mechanical removal of cells from the test surface and/or a function of differences in variability of cells within a population remains to be determined.

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